Quantitative PCR

- END PIONT product analysis

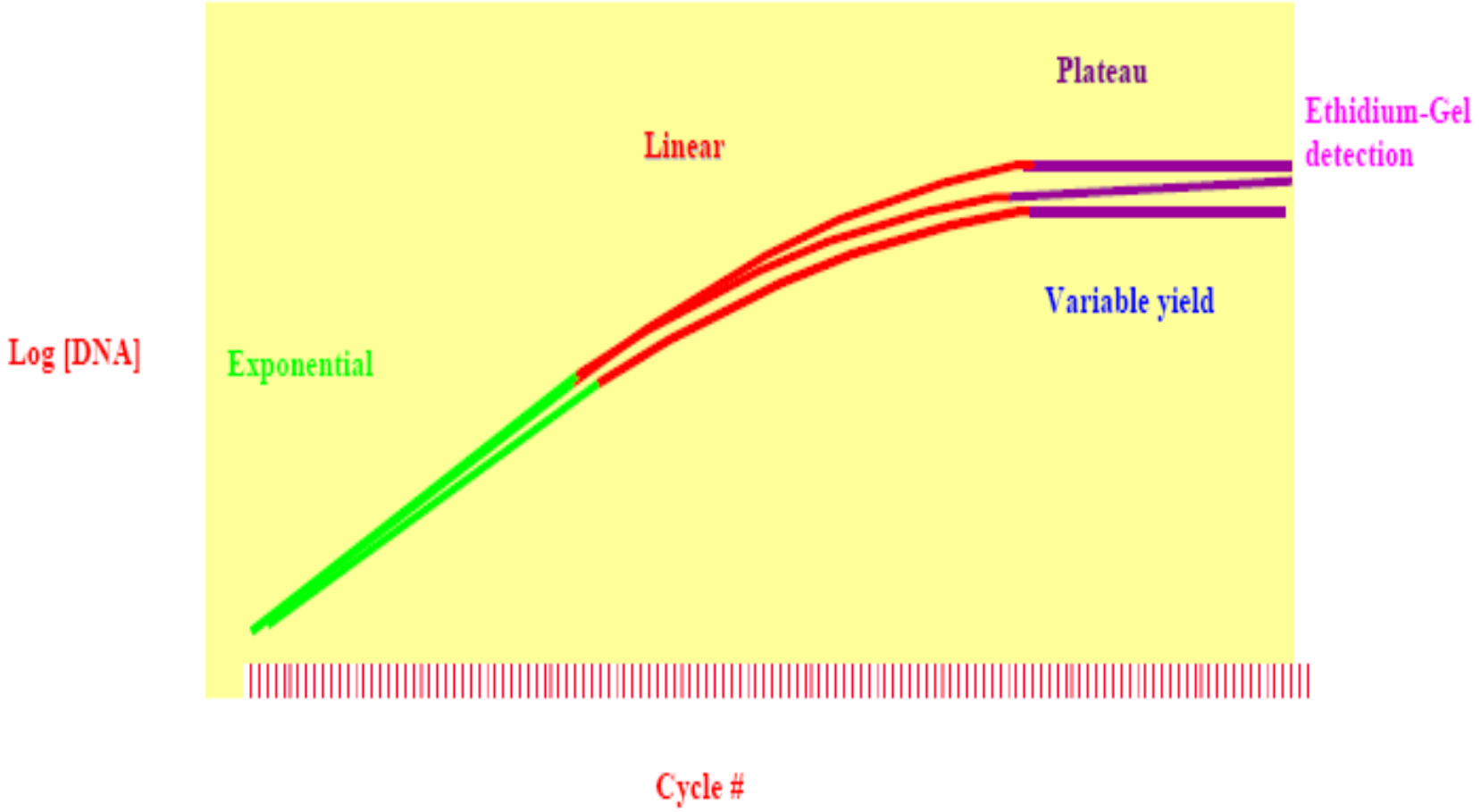
Traditional method that uses Agarose gels for detection of PCR amplification at the final phase or end-point of the PCR reaction

A basic PCR run can be broken up into three phases

- Exponential: Exact doubling of product is accumulating at **every cycle** (assuming 100% reaction efficiency). The reaction is very specific and precise.
- Linear (High Variability): The reaction components are being consumed, the reaction is slowing, and products are starting to degrade.
- Plateau (End-Point Gel detection for traditional methods):

  The reaction has stopped, no more products are being made and if left long enough, the PCR products will begin to degrade

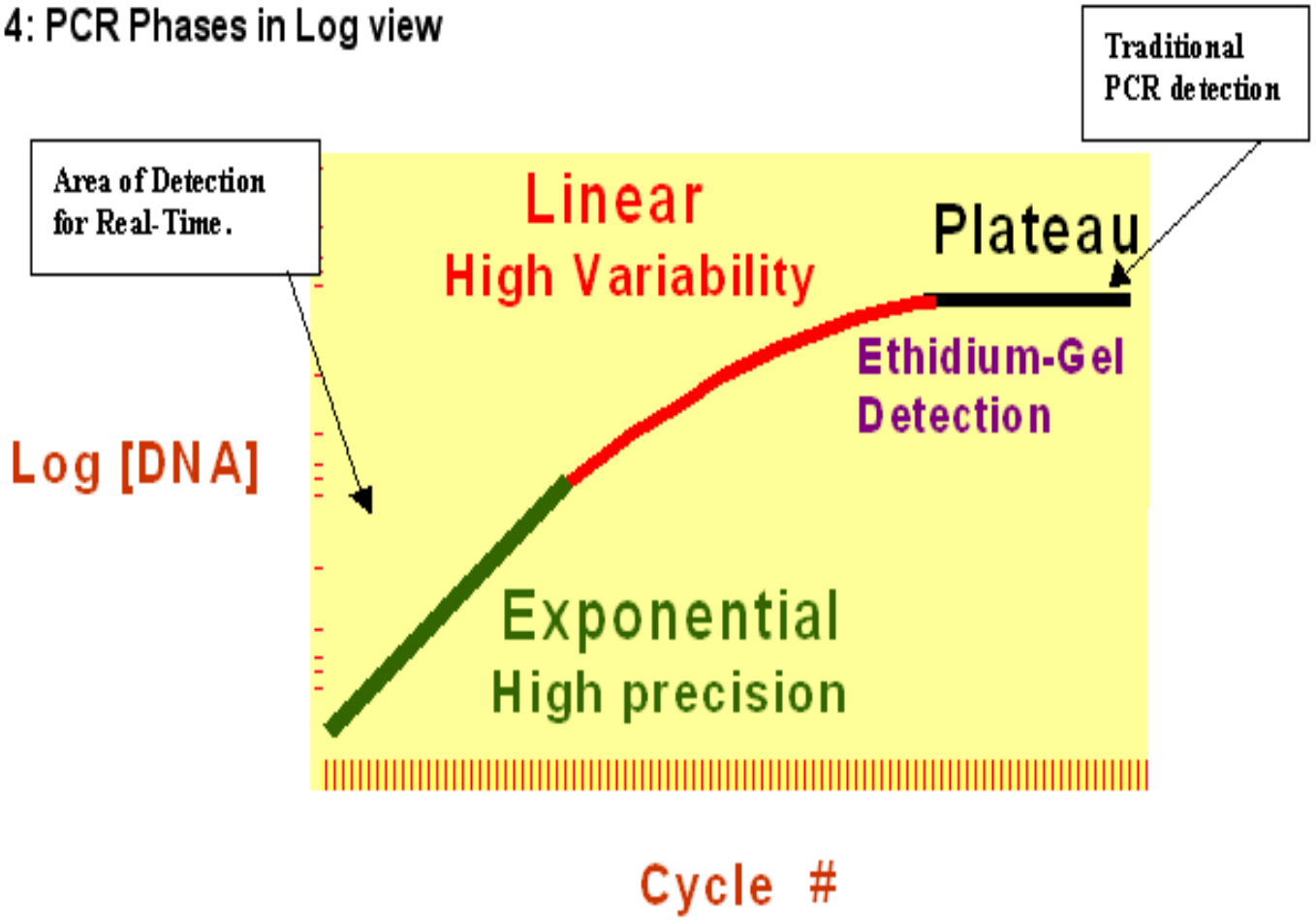
PCR Phases


Plateau
Ethidium-Gel detection
Linear
Variable yield
Log [DNA]
Exponential
Cycle #

Limitations of End-Point PCR

- Time consuming (Post PCR processing)
- Poor Precision (Results are based on size discrimination)
- Low sensitivity (The end point is variable from sample to sample. While gels may not be able to resolve these variabilities in yield).
- Results are not expressed as numbers
- Non - Automated

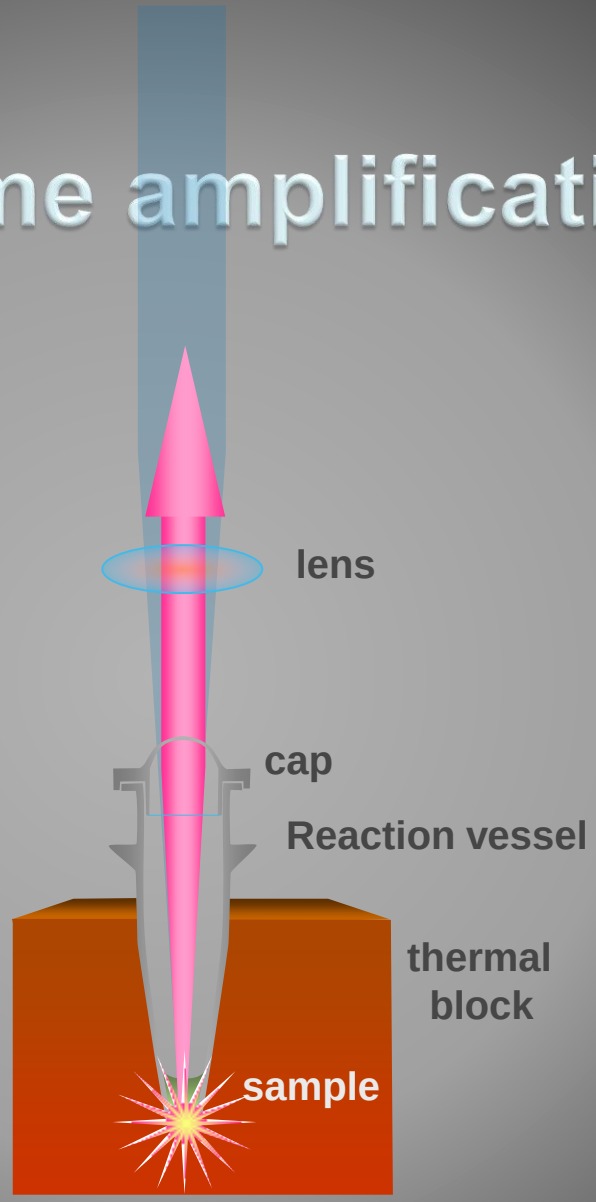
Figure 4: PCR Phases in Log view



The Evolution of PCR to Real-Time

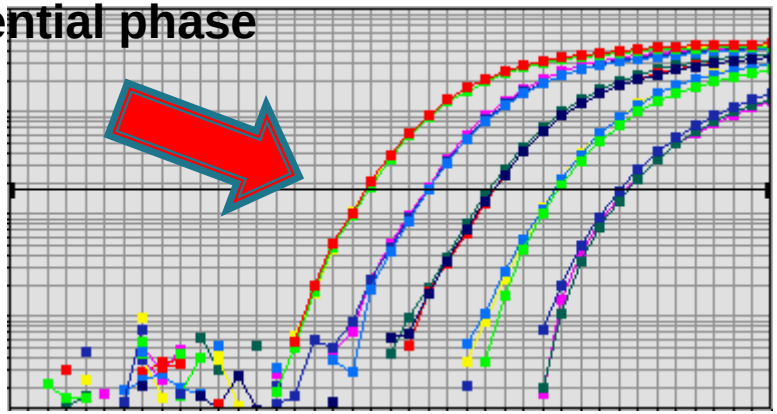
- From detection at the **end-point** of the reaction to detection **while the reaction is occurring**

What is real-time amplification?



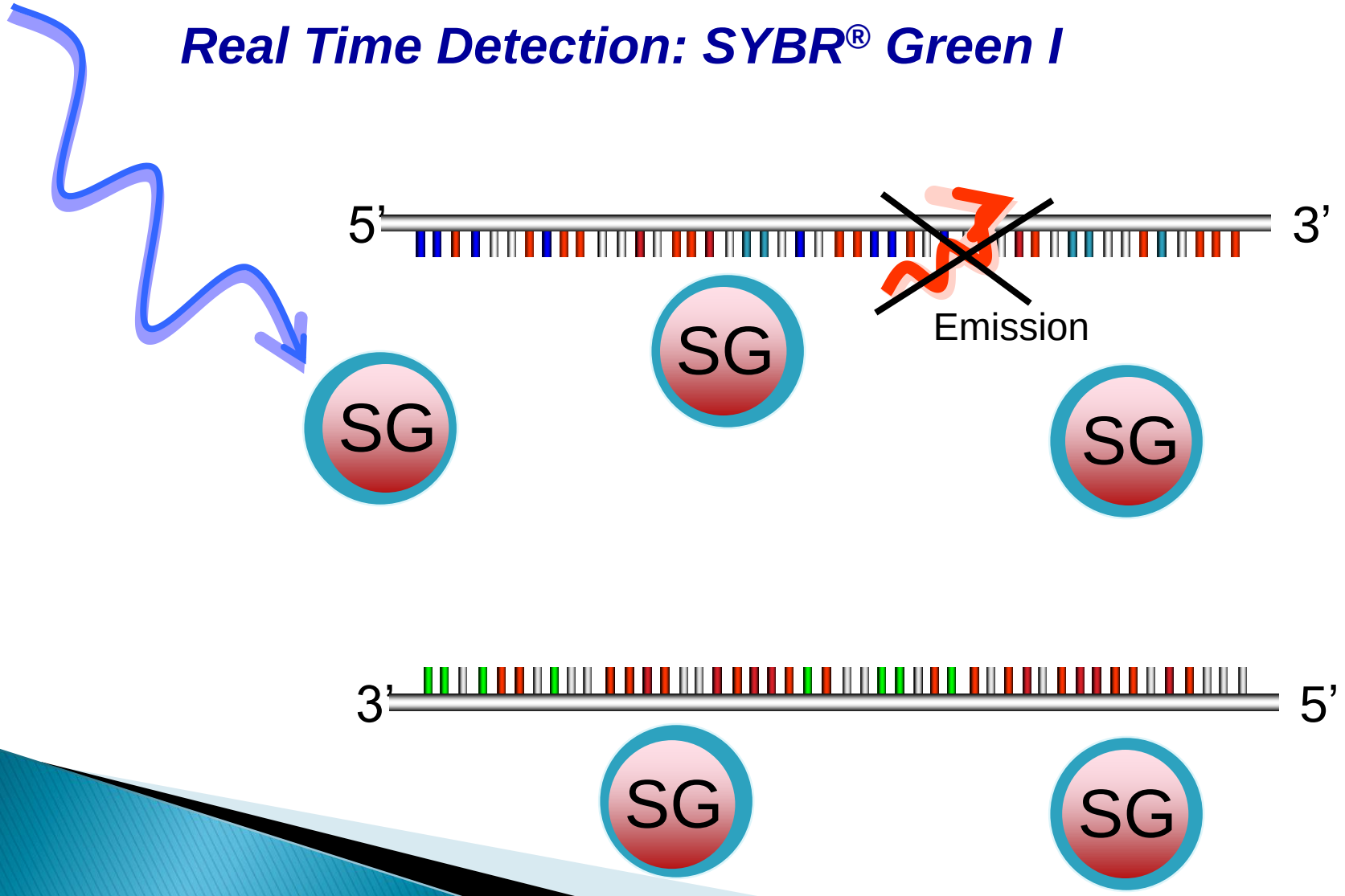
Plateau effect

Exponential phase

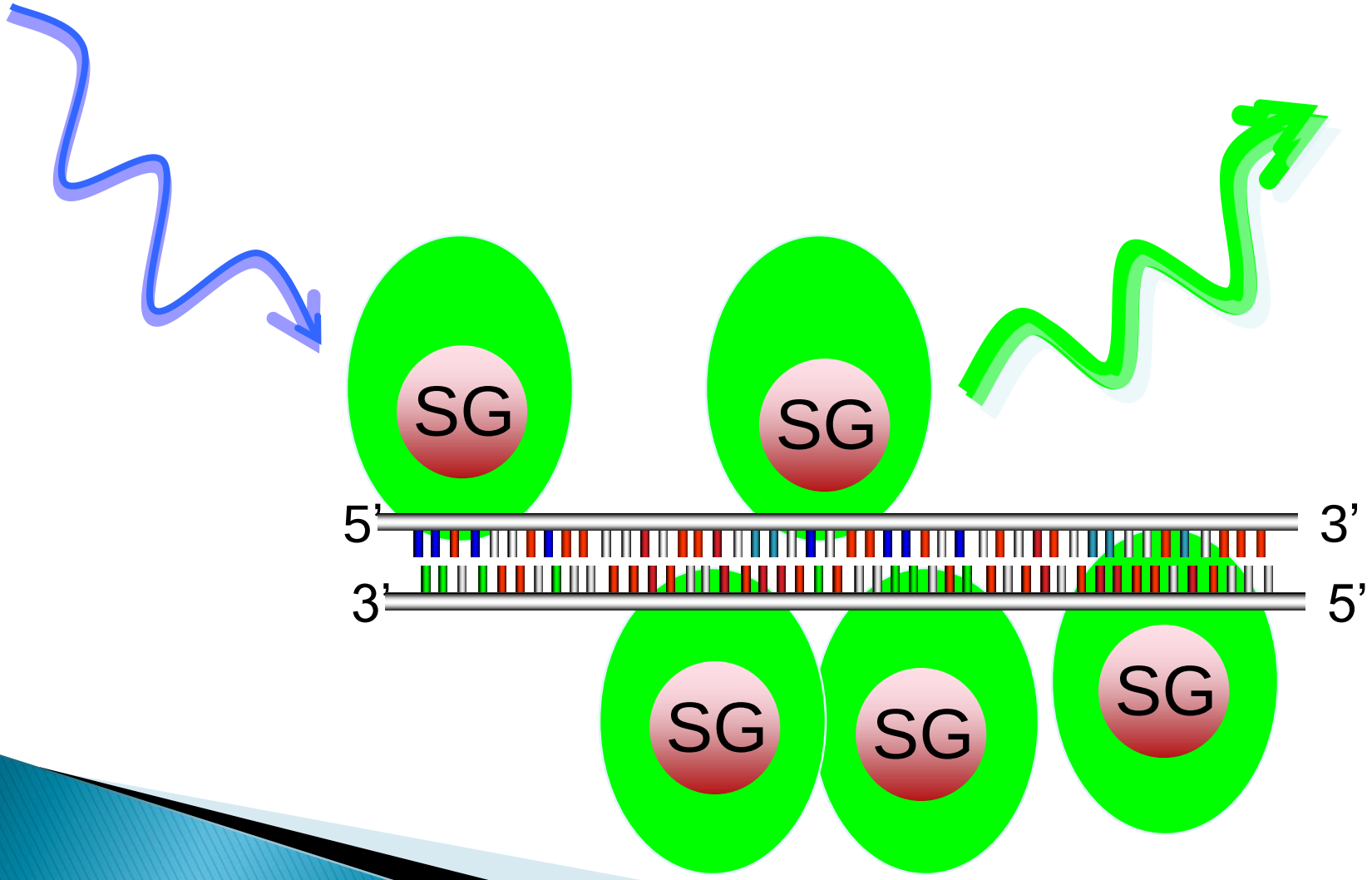


Rn

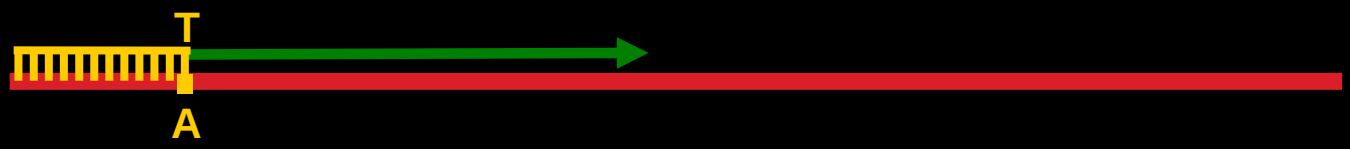
Real Time Detection: SYBR® Green I



Real Time Detection: SYBR® Green I

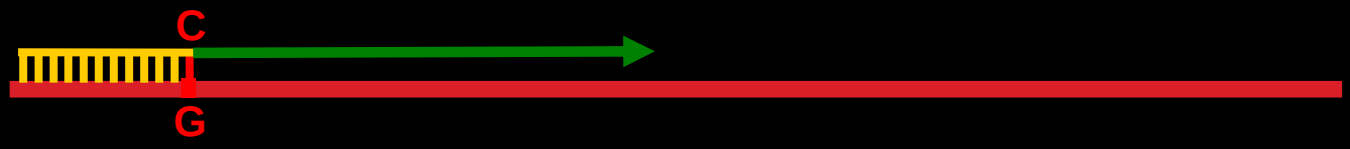


Wt primer + Wt Sample DNA: Signal Production



Wt primer + Mutant Sample DNA: No Signal Production

Mutant primer + Mutant Sample DNA: Signal Production



Mutant primer + Wt Sample DNA: No Signal Production

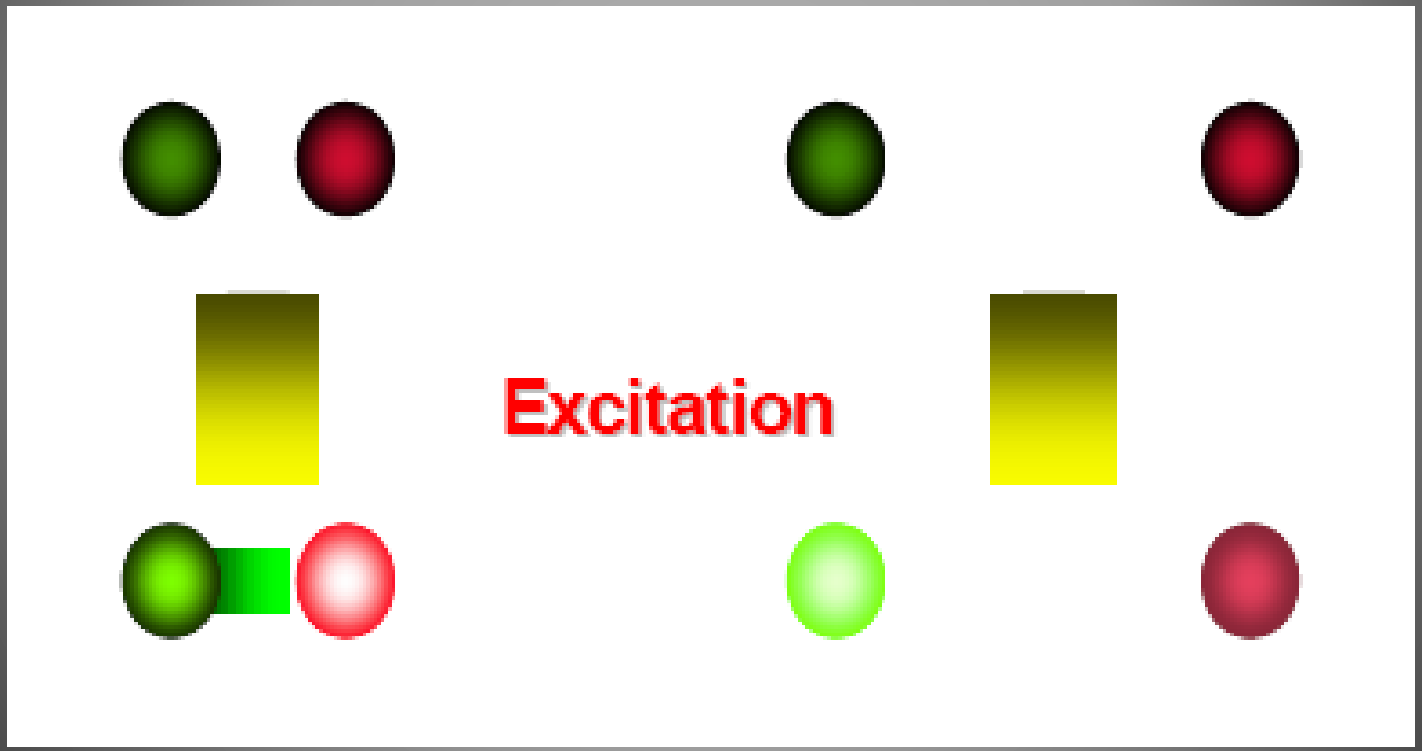
Real Time Detection: SYBR® Green I

- Positive and Negative Points
  - Easy to plan and perform
  - Cheap
  - Needs optimization
  - False signal from primer dimer and non-specific products

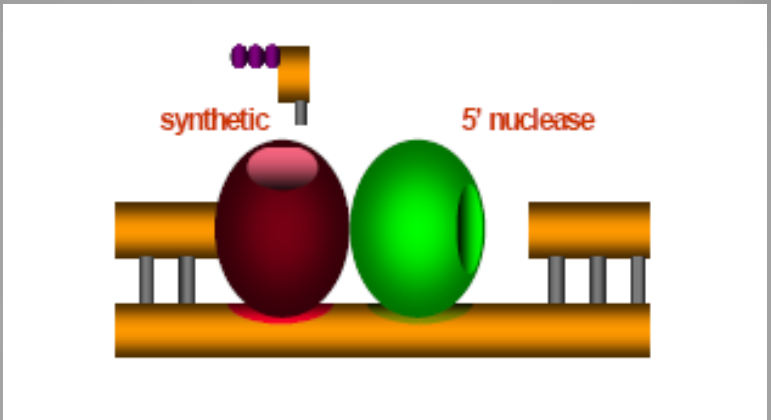
Chemistries:

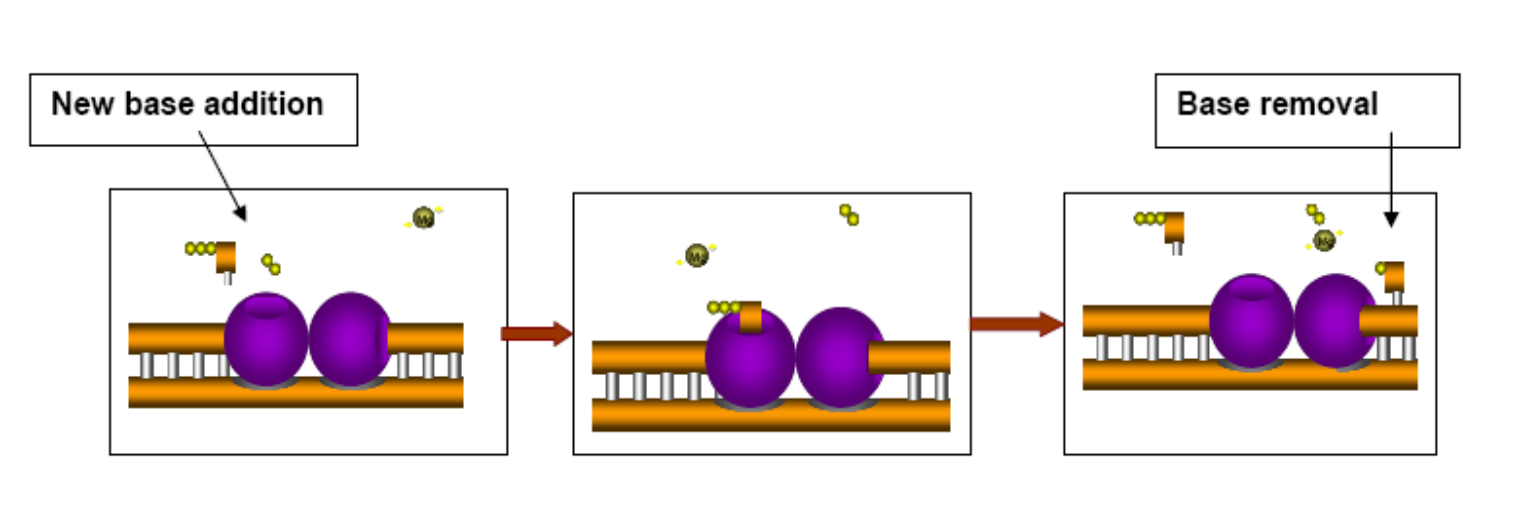
- SYBR Green
- TaqMan Probes

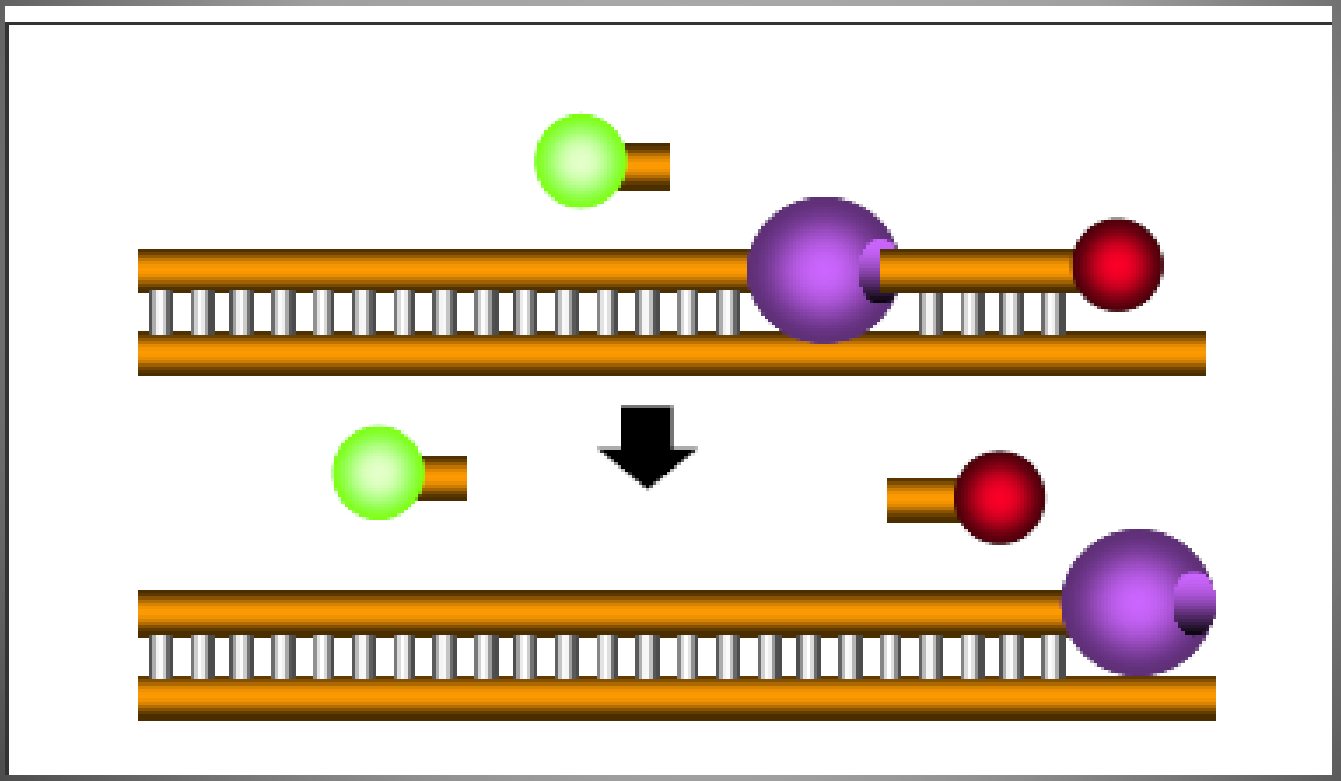
FRET (Fluorescent Resonance Energy Transfer)



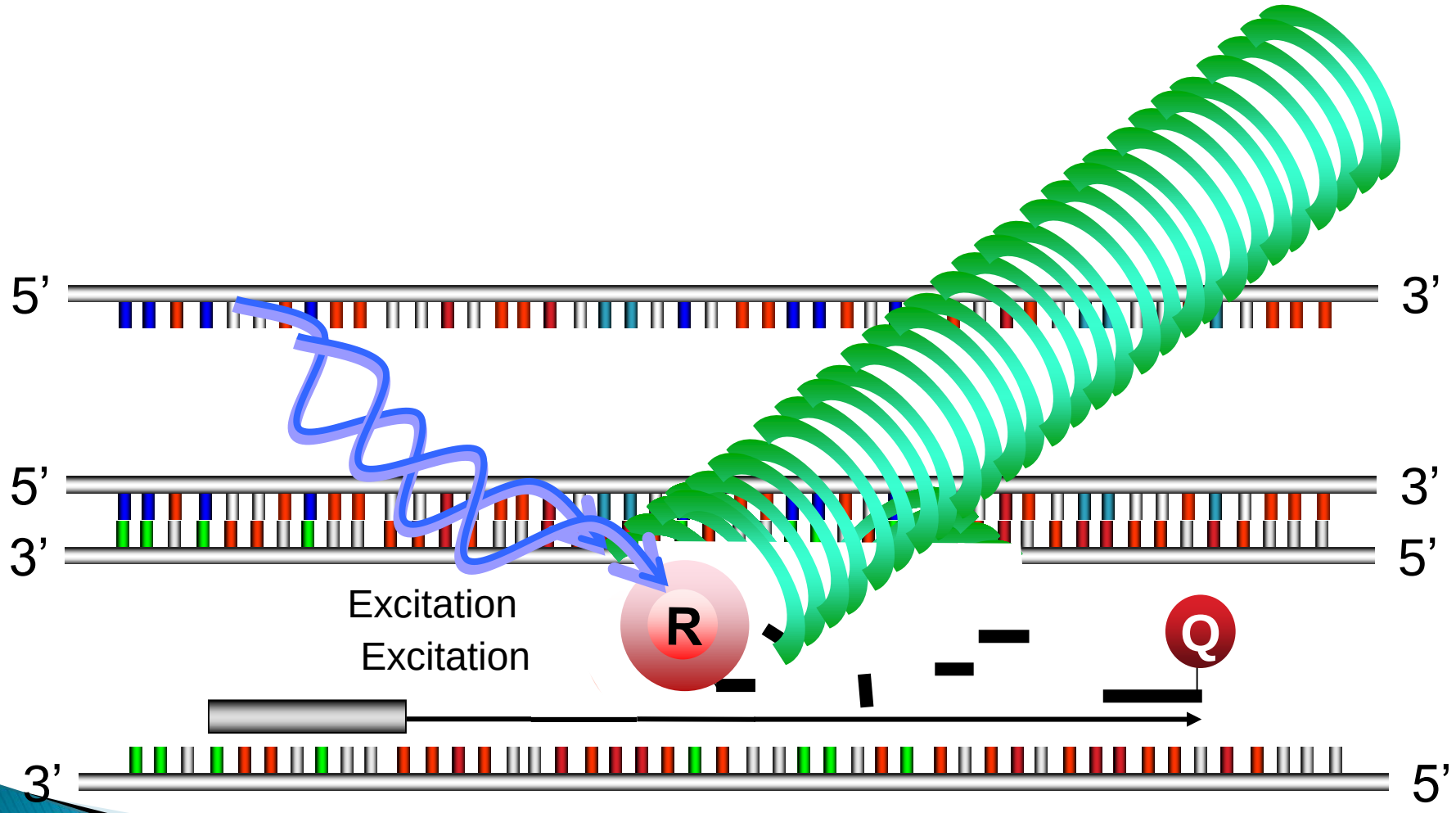
5′ Exo-Nuclease Activity of Taq Polymerase







Taq Man probes probes



Primer design

Primers

- Primers should be 20–24 bases in length.
- Short amplicons work best. Amplicon size should not exceed 300bp.
- Keep the GC content in the range of 20–80% (ideally 40–60%).
- Avoid including long sequences of identical nucleotides.
- T_m should be 55 to 60°C.
- Place the forward and reverse primers as close as possible to the probe without overlapping it.

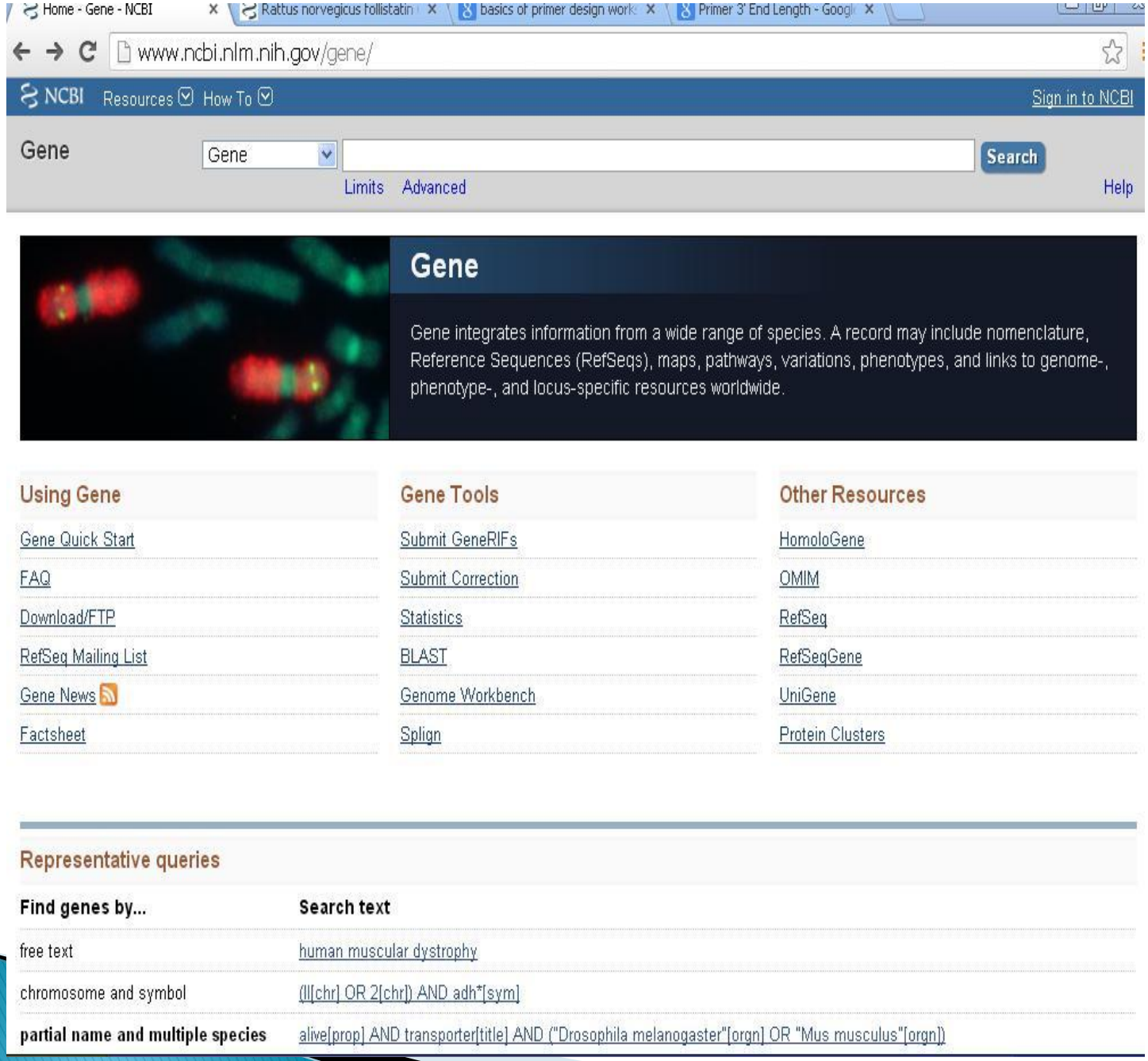
Probe design

Probes

- Probe should be 20–30 bases in length.
- Avoid including long sequences of identical nucleotides.
- The 5' end of a probe cannot be a guanosine residue. A guanosine residue adjacent to the reporter dye will quench some of the reporter fluorescence even after cleavage.
- The melting temperature (T_m) of the primers should be 65 to 67°C. The probe T_m should be approximately 10°C higher than the primer T_m.
- The reporter dye should be located on the 5' end and the quencher dye should be on 3' end.

Primer design

- If oligo dT primers are used in RT, the real-time PCR primers should be picked from the 3’ region of a gene sequence to gain maximum assay sensitivity.
- If random priming strategy is adopted, real-time PCR primers should be picked close to the 5’ end of the target sequence for maximum sensitivity in real-time PCR.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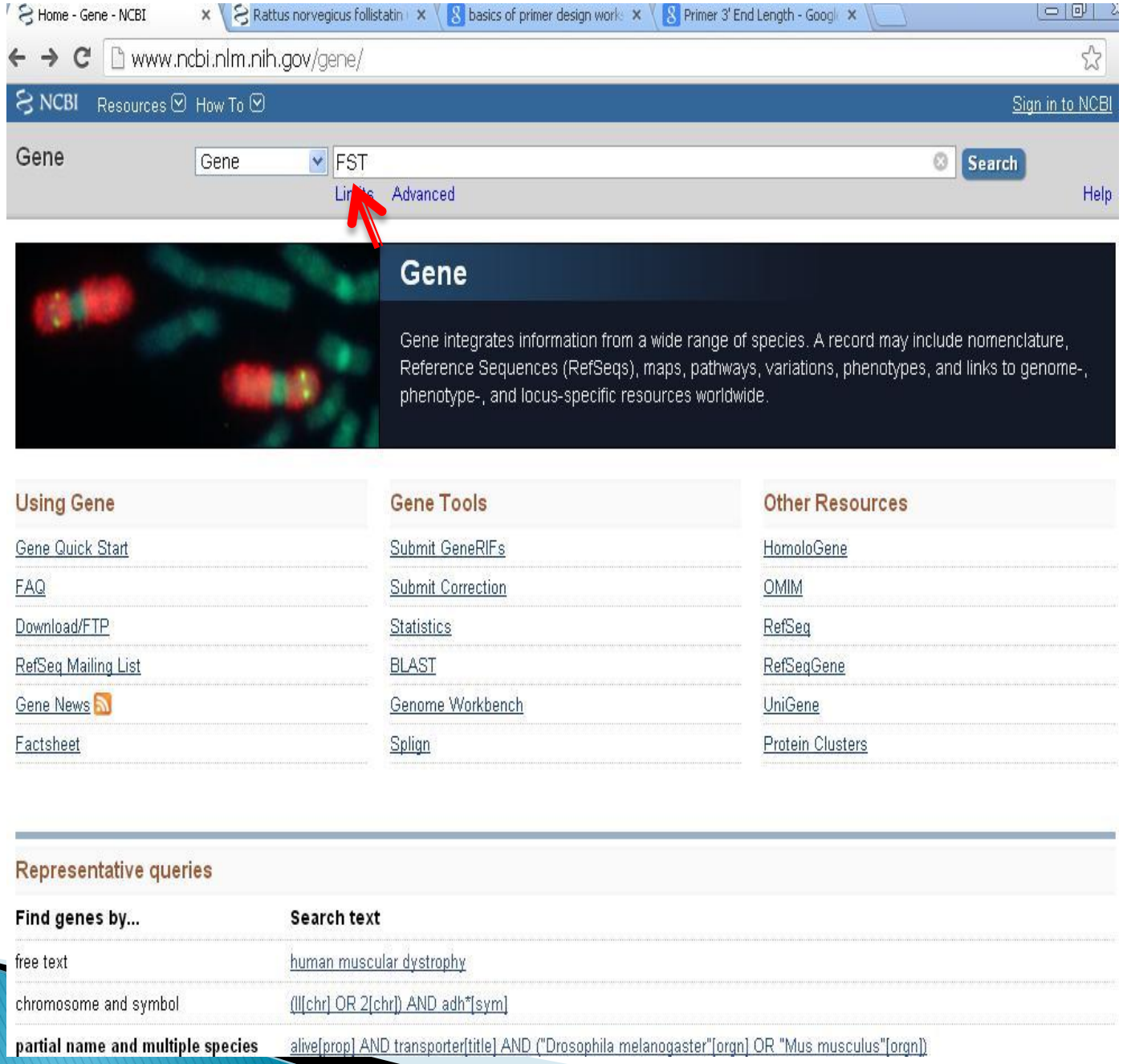
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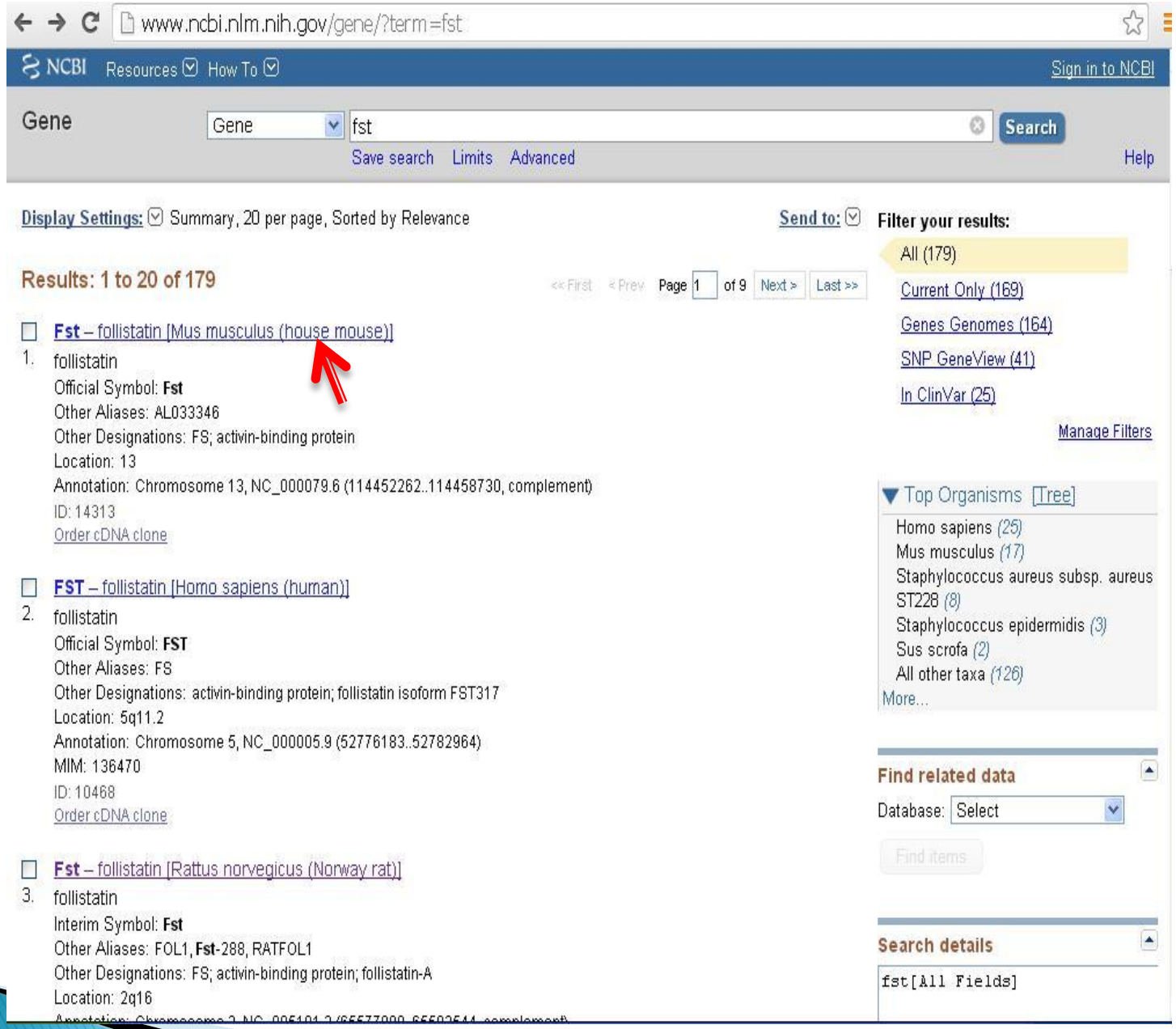
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<< First < Prev Page 1 of 9 Next > Last >>
Fst – follistatin [Mus musculus (house mouse)]
1. follistatin
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Other Aliases: AL033346
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Other Aliases: FOL1, Fst-288, RATFOL1
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Staphylococcus epidermidis (3)
Sus scrofa (2)
All other taxa (126)
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Fst follistatin [*Rattus norvegicus* (Norway rat)]

Gene ID: 24373, updated on 5-Nov-2013

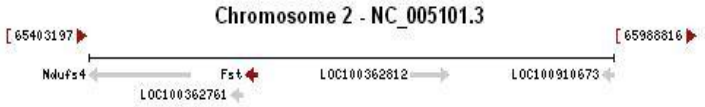
Summary

Symbol	Fst provided by RGD
Full Name	follistatin provided by RGD
Primary source	RGD:2633
See related	Ensembl:ENSRNOG00000011631
Gene type	protein coding
RefSeq status	PROVISIONAL
Organism	Rattus norvegicus
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Sciurognathi; Muroidea; Muridae; Murinae; Rattus
Also known as	FOL1; Fst-288; RATFOL1
Summary	binds activin and inhibits activin-mediated signaling pathways [RGD, Feb 2006]

Genomic context

Location: 2q16 See Fst in MapViewer

Sequence: Chromosome: 2; NC_005101.3 (65577888..65583544, complement)



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- General protein information
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Related information

- 3D structures
- BioProjects
- BioSystems
- Conserved Domains
- Full text in PMC
- Full text in PMC_nucleotide
- Gene neighbors
- Genome

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Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia; Sciurognathi; Muroidea; Muridae; Murinae; Rattus

Also known as FOL1; Fst-288; RATFOL1

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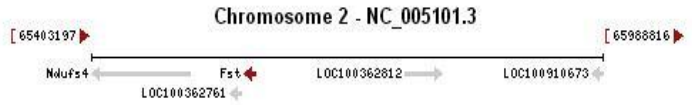

Genomic regions, transcripts, and products

Genomic Sequence NC_005101 chromosome 2 reference Rnor_5.0 Primary Assembly Go to reference sequence details

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Bibliography

Related articles in PubMed

1. Proliferation of rat small hepatocytes requires follistatin expression.
   Ooe H, *et al.* J Cell Physiol, 2012 Jun. PMID 21826650.
2. Structural basis for the inhibition of activin signalling by follistatin.
   Harrington AE, *et al.* EMBO J, 2006 Mar 8. PMID 16482217.
3. Follistatin attenuates early liver fibrosis: effects on hepatic stellate cell activation and hepatocyte apoptosis.
   Patella S, *et al.* Am J Physiol Gastrointest Liver Physiol, 2006 Jan. PMID 16123203.
4. Crystal structures of the heparan sulfate-binding domain of follistatin. Insights into ligand binding.

Additional links

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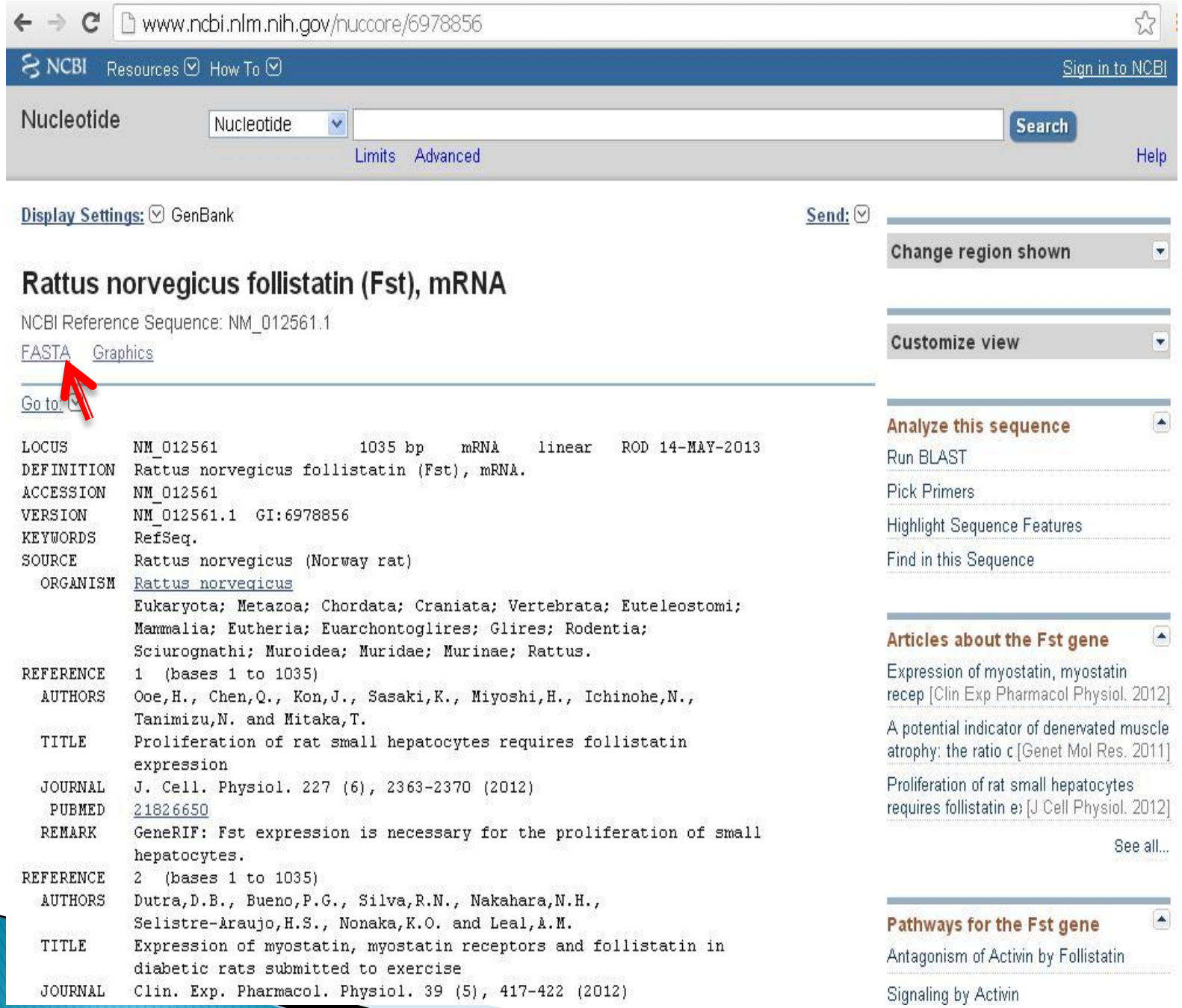
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REFERENCE 1 (bases 1 to 1035)
AUTHORS Ooe,H., Chen,Q., Kon,J., Sasaki,K., Miyoshi,H., Ichinohe,N.,
Tanimizu,N. and Mitaka,T.
TITLE Proliferation of rat small hepatocytes requires follistatin
expression
JOURNAL J. Cell. Physiol. 227 (6), 2363-2370 (2012)
PUBMED 21826650
REMARK GeneRIF: Fst expression is necessary for the proliferation of small
hepatocytes.
REFERENCE 2 (bases 1 to 1035)
AUTHORS Dutra,D.B., Bueno,P.G., Silva,R.N., Nakahara,N.H.,
Selistre-Araujo,H.S., Nonaka,K.O. and Leal,A.M.
TITLE Expression of myostatin, myostatin receptors and follistatin in
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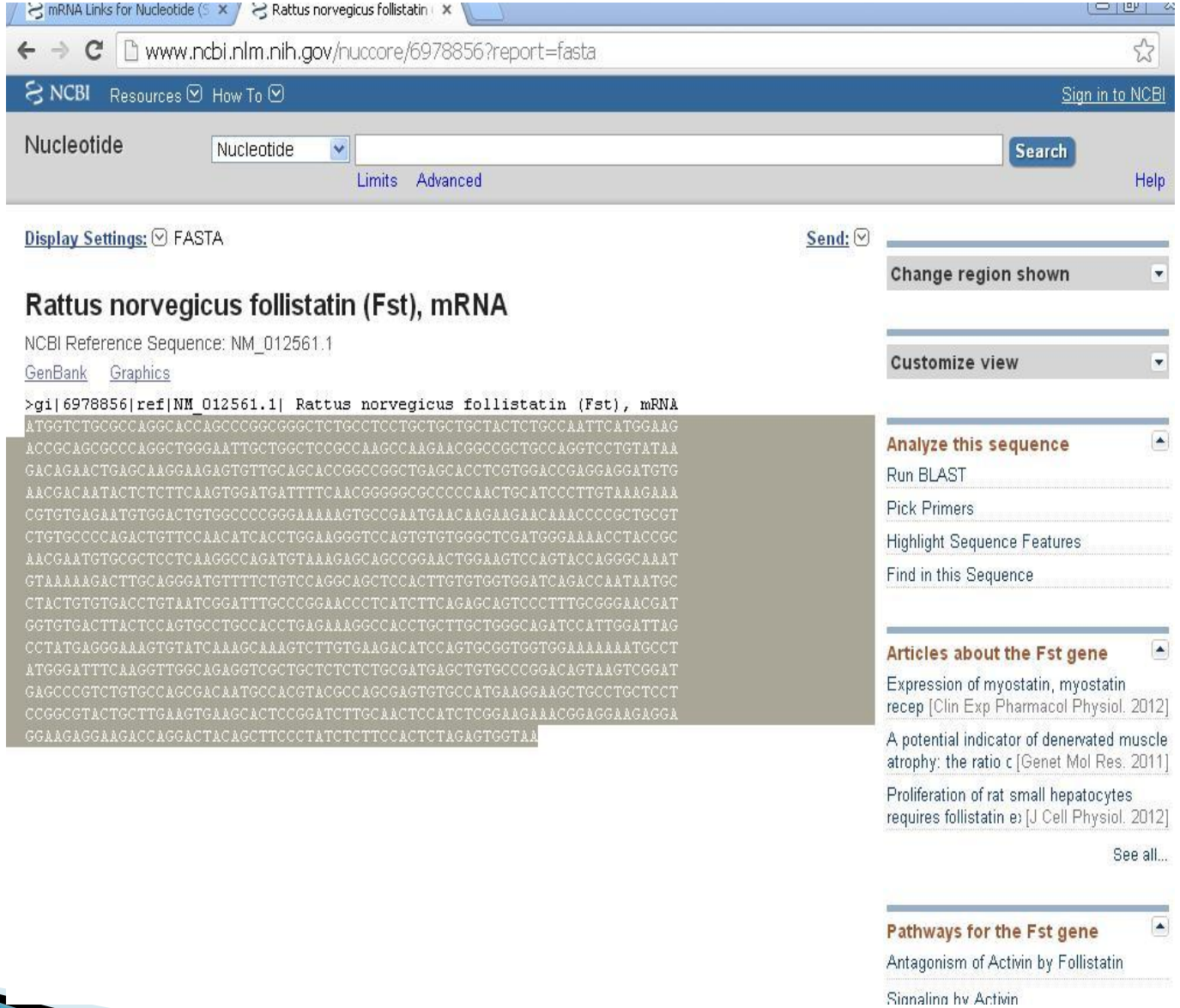
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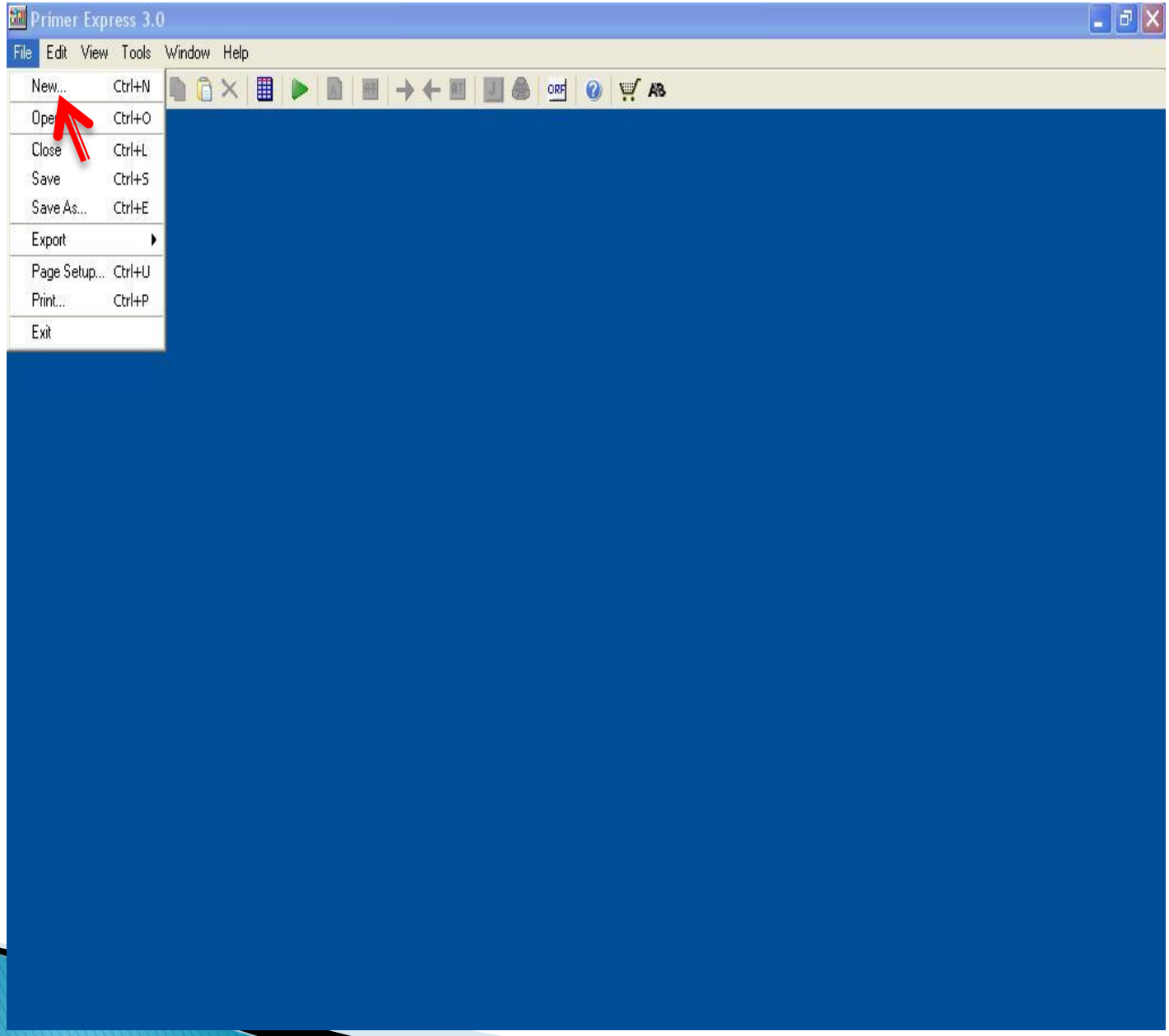
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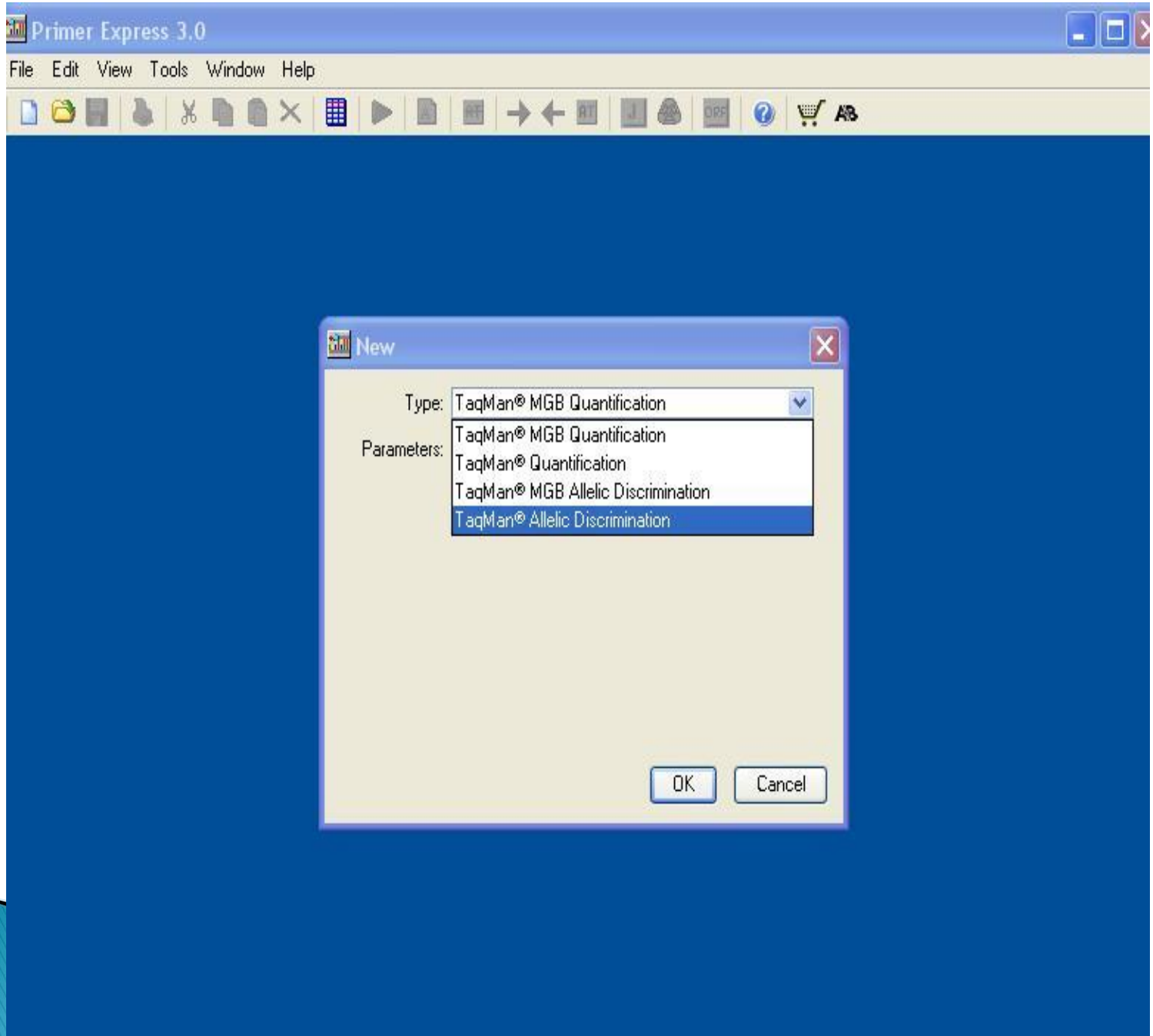
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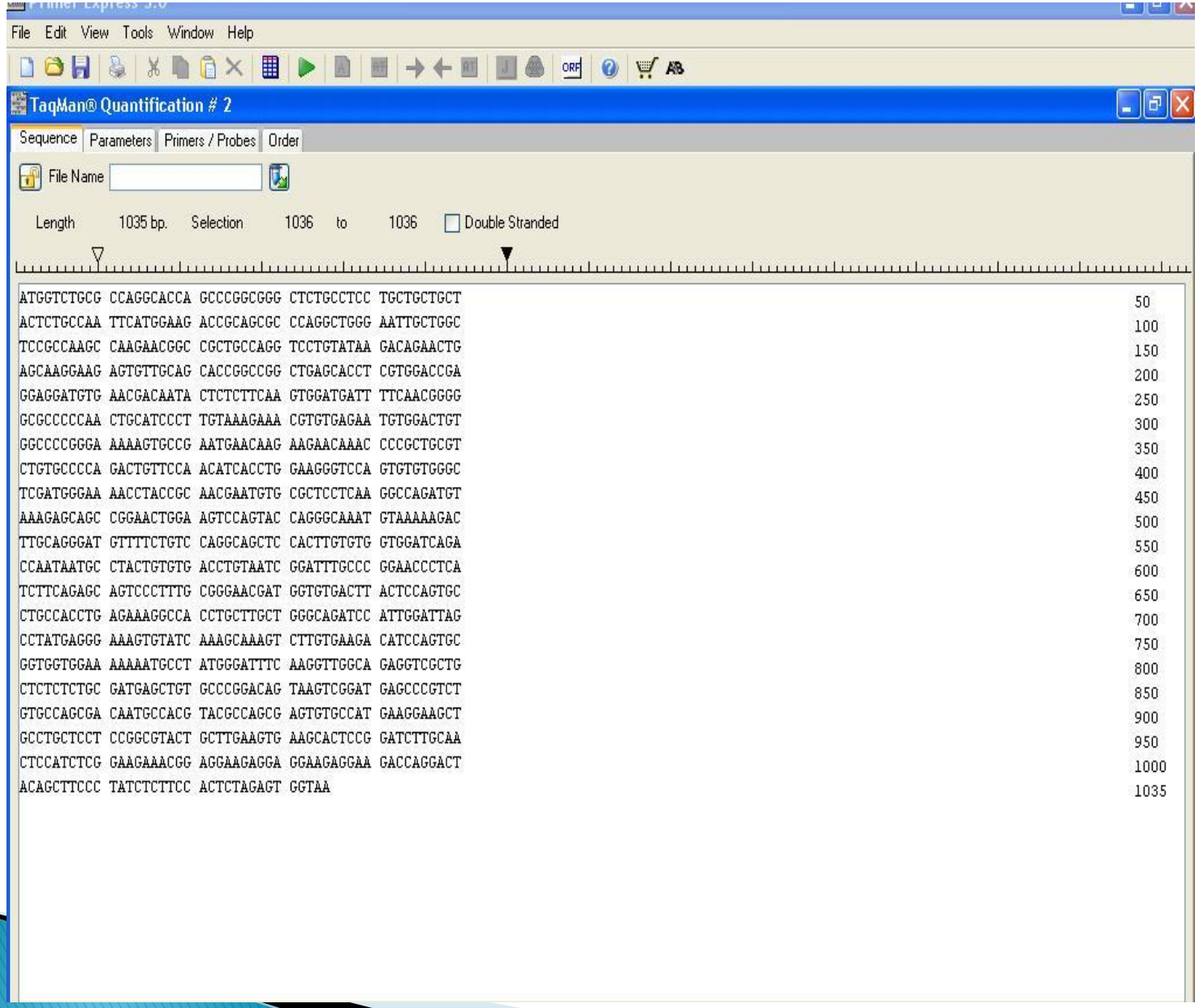
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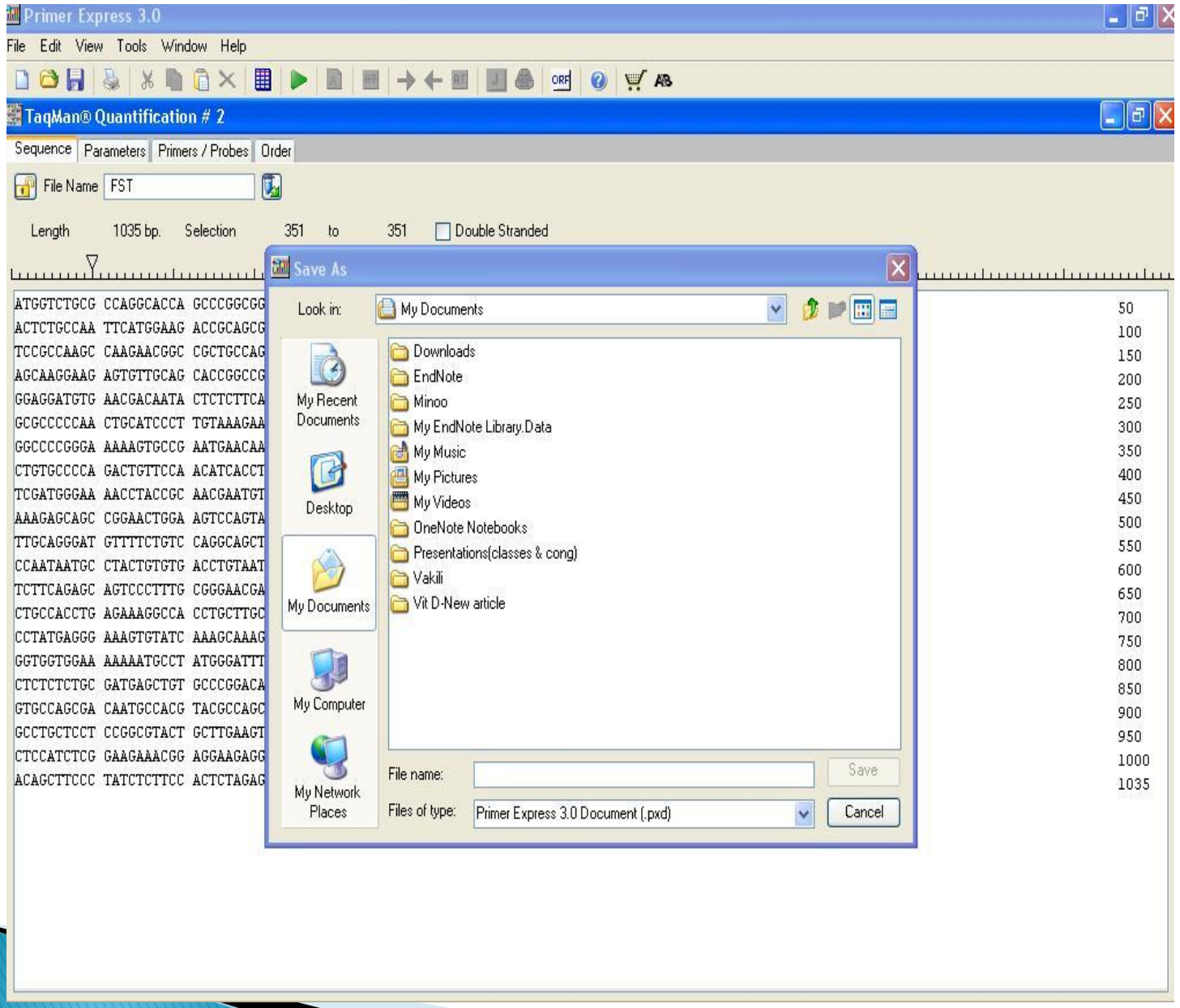
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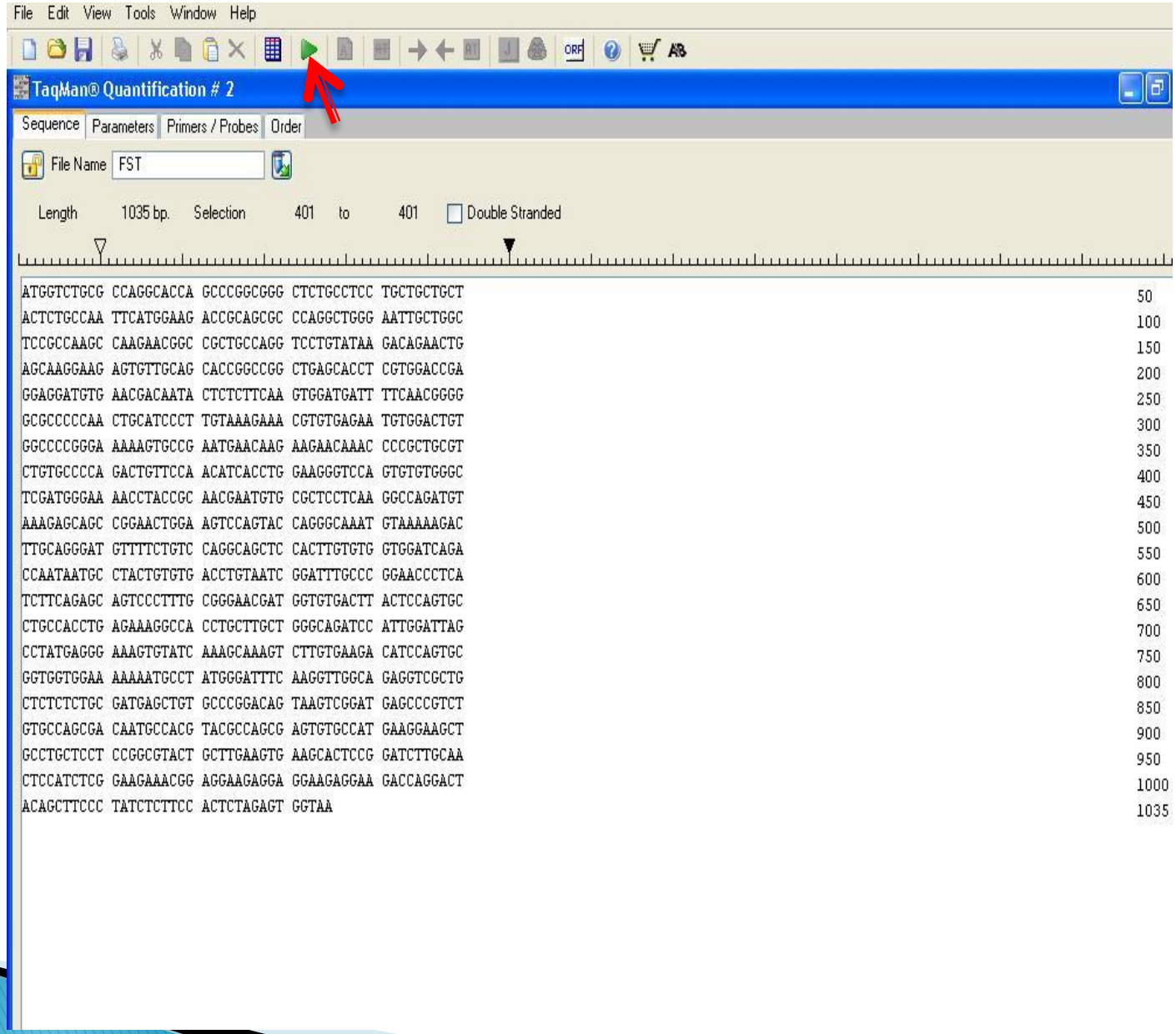
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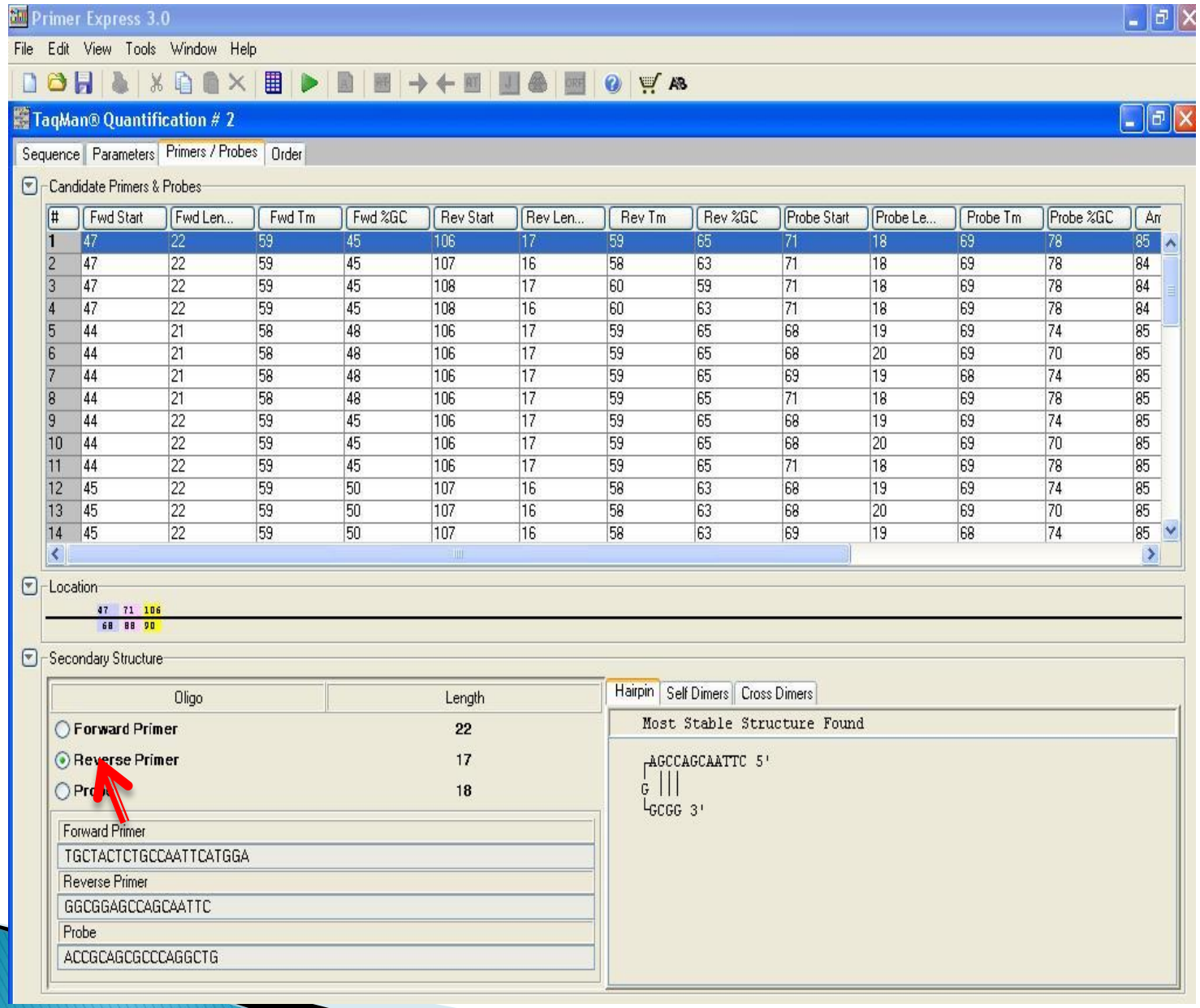
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14 45 22 59 50 107 16 58 63 69 19 68 74 85
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Oligo Length
Forward Primer 22
Reverse Primer 17
Probe 18
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Probe
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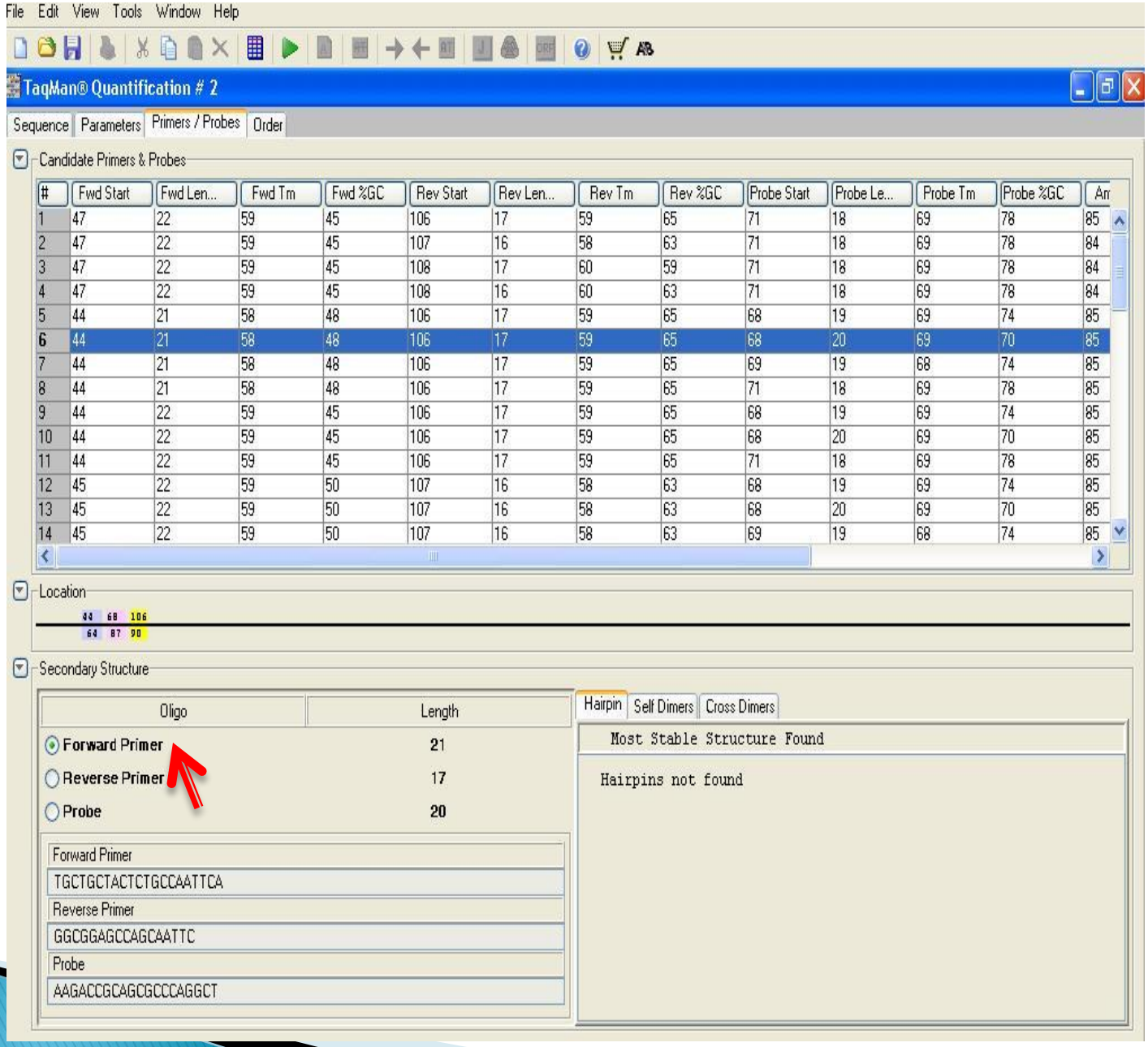
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Oligo Length
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Reverse Primer 17
Probe 20
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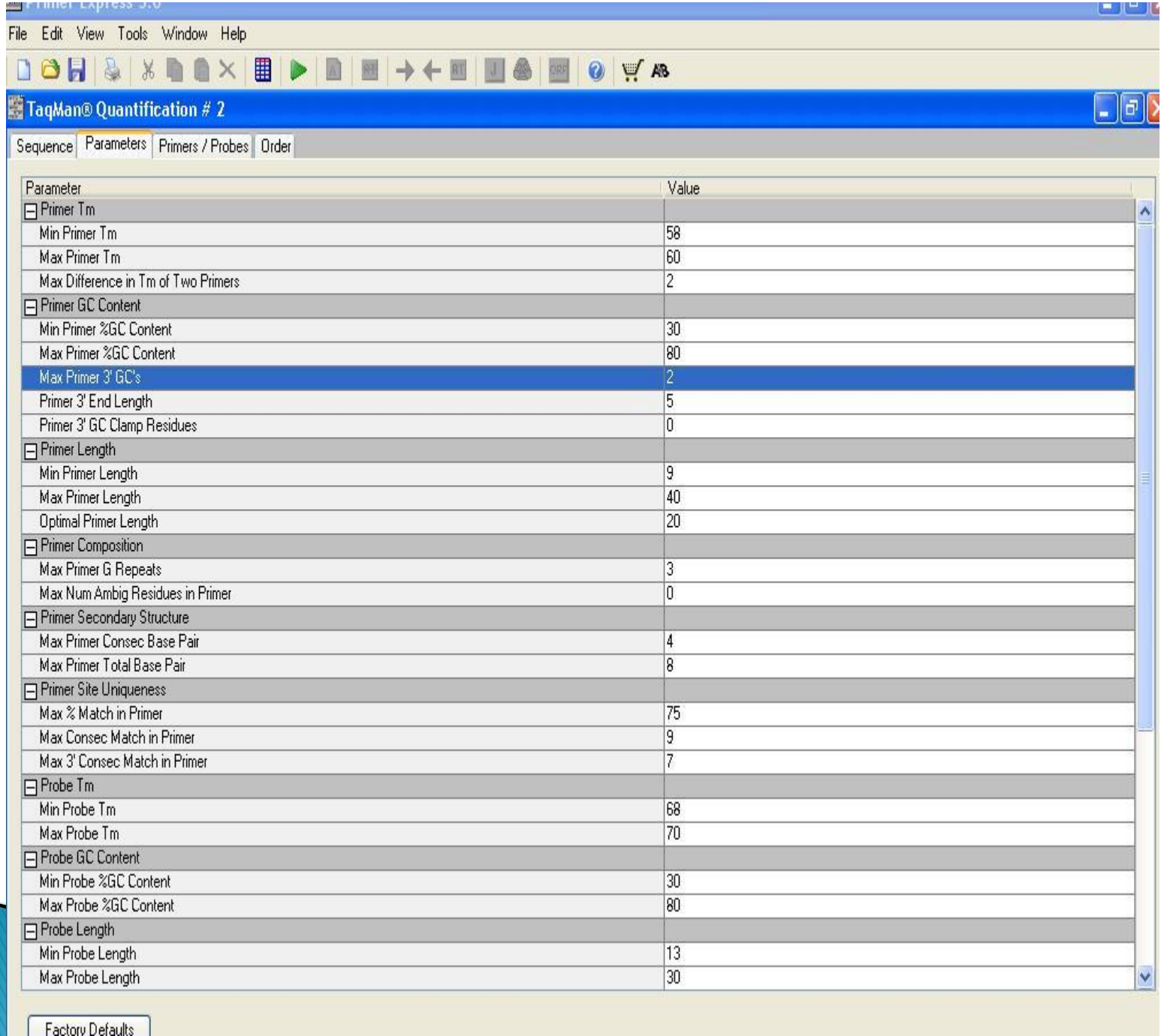
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Order
Parameter
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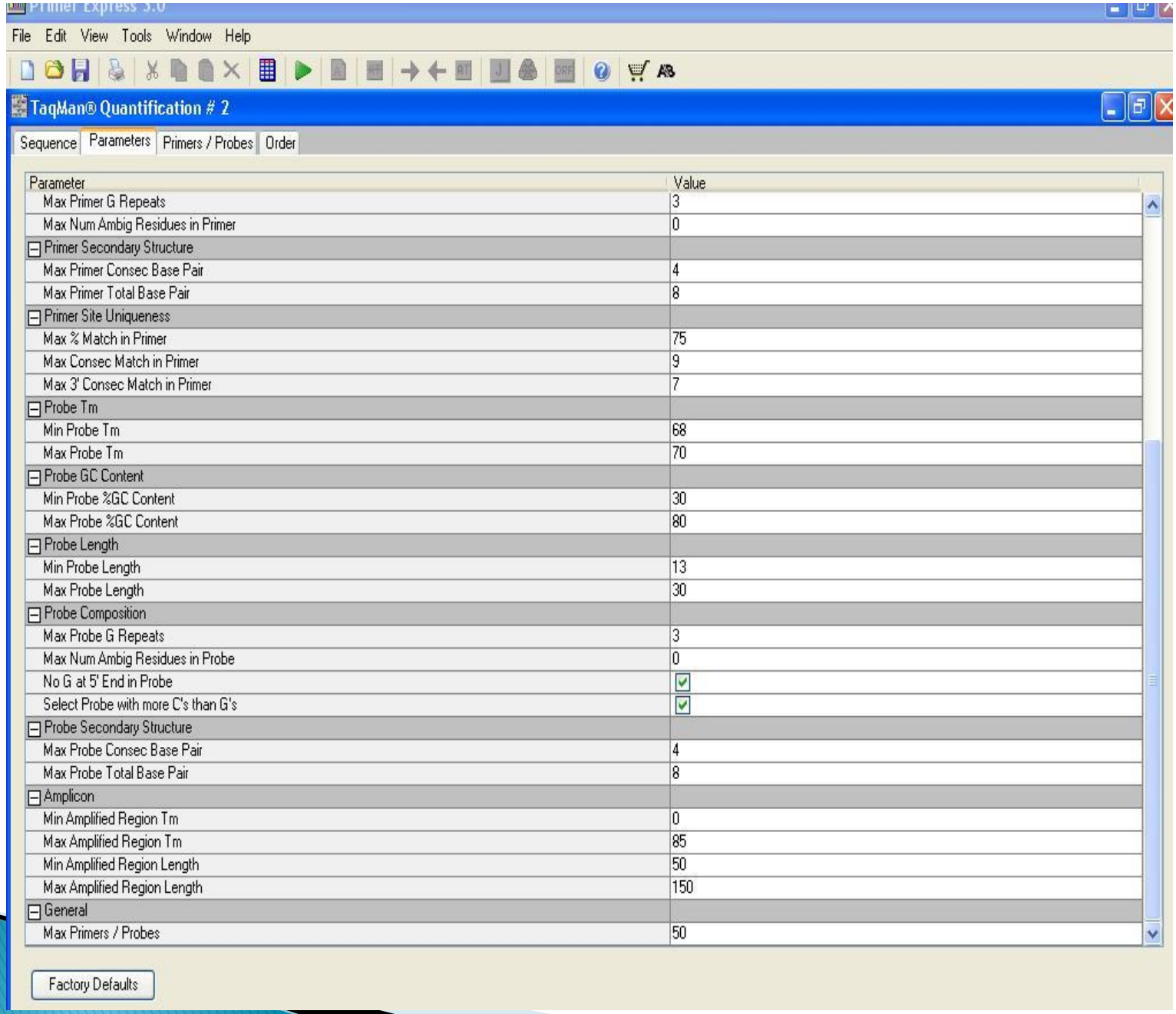
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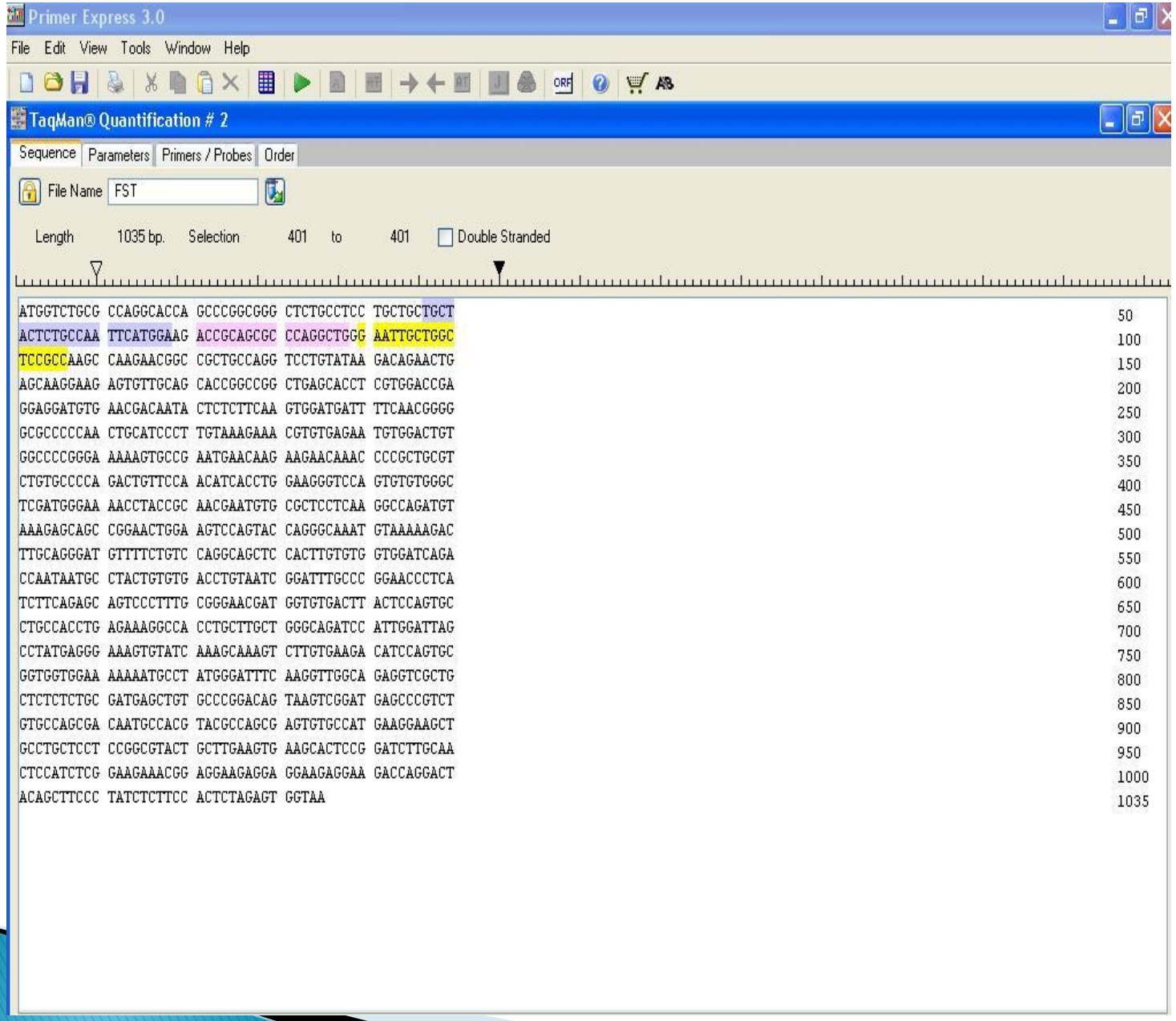
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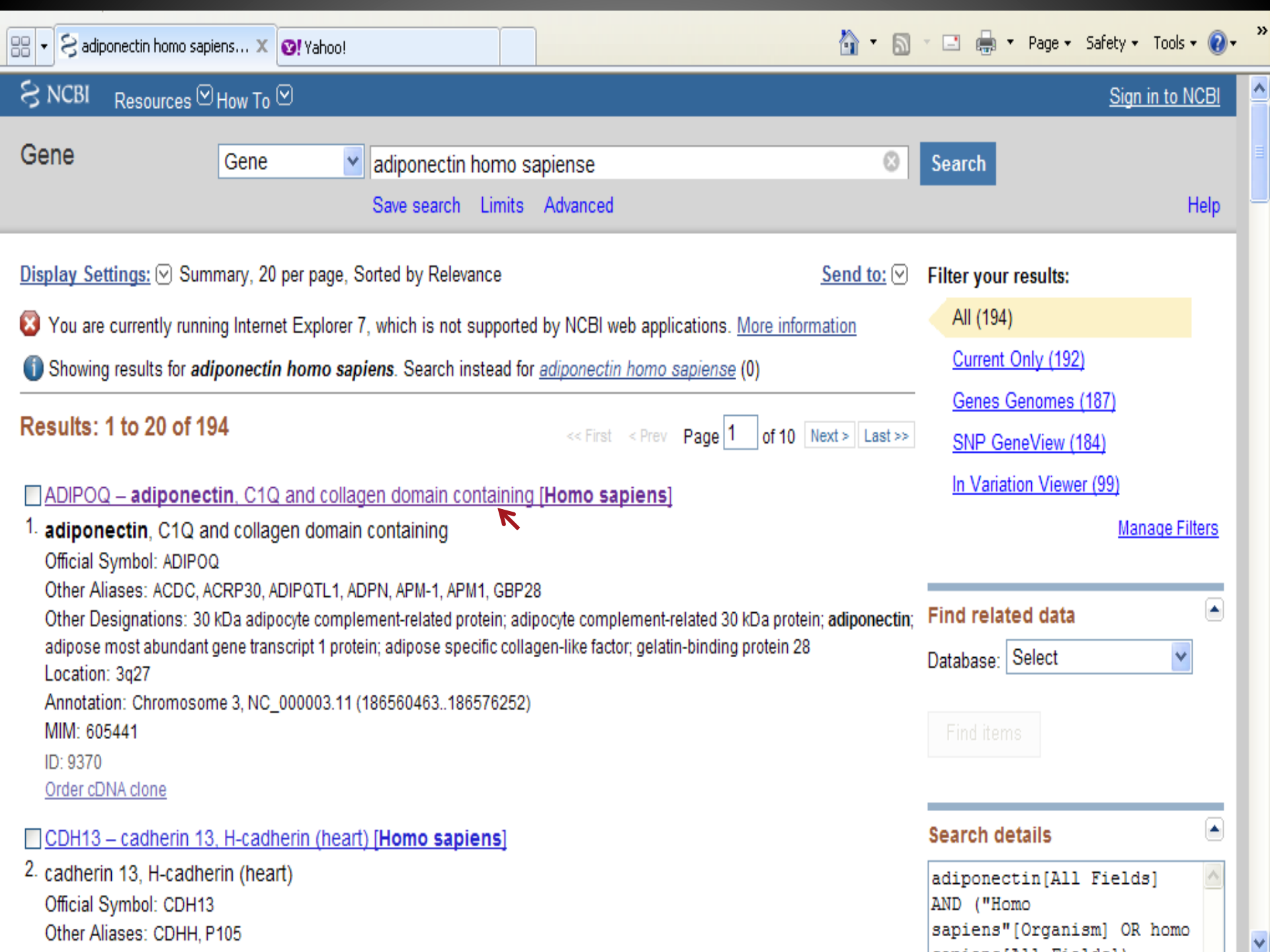
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Page 1 of 10
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ADIPOQ – adiponectin, C1Q and collagen domain containing [Homo sapiens]
1. adiponectin, C1Q and collagen domain containing
Official Symbol: ADIPOQ
Other Aliases: ACDC, ACRP30, ADIPQTL1, ADPN, APM-1, APM1, GBP28
Other Designations: 30 kDa adipocyte complement-related protein; adipocyte complement-related 30 kDa protein; adiponectin; adipose most abundant gene transcript 1 protein; adipose specific collagen-like factor; gelatin-binding protein 28
Location: 3q27
Annotation: Chromosome 3, NC_000003.11 (186560463..186576252)
MIM: 605441
ID: 9370
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CDH13 – cadherin 13, H-cadherin (heart) [Homo sapiens]
2. cadherin 13, H-cadherin (heart)
Official Symbol: CDH13
Other Aliases: CDHH, P105
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Database: Select
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AND ("Homo
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ADIPOQ adiponectin, C1Q and collagen domain containing [*Homo sapiens*]

Gene ID: 9370, updated on 17-Feb-2013

Summary

Official Symbol	ADIPOQ provided by HGNC
Official Full Name	adiponectin, C1Q and collagen domain containing provided by HGNC
Primary source	HGNC:13633
See related	Ensembl:ENSG00000181092; HPRD:05671; MIM:605441; Vega:OTTHUMG00000156521
Gene type	protein coding
RefSeq status	REVIEWED
Organism	Homo sapiens
Lineage	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as	ACDC; ADPN; APM1; APM-1; GBP28; ACRP30; ADIPQTL1
Summary	This gene is expressed in adipose tissue exclusively. It encodes a protein with similarity to collagens X and VIII and complement factor C1q. The encoded protein circulates in the plasma and is involved with metabolic and hormonal processes. Mutations in this gene are associated with adiponectin deficiency. Multiple alternatively spliced variants, encoding the same protein, have been identified. [provided by RefSeq, Apr 2010]

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Summary
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Related information

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ADIPOQ adiponectin, C1... Yahoo!
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30 kDa adipocyte complement-related protein
adipocyte complement-related 30 kDa protein
adipose most abundant gene transcript 1 protein

NCBI Reference Sequences (RefSeq)

RefSeqs maintained independently of Annotated Genomes

These reference sequences exist independently of genome builds. Explain

Genomic

1. **NG_021140.1 RefSeqGene**

Range	5001..20790
Download	GenBank, FASTA, Sequence Viewer (Graphics)

mRNA and Protein(s)

1. NM_001177800.1 → NP_001171271.1 **adiponectin precursor**
Status: REVIEWED

Description	Transcript Variant: This variant (1) represents the longer transcript. Both variants 1 and 2 encode the same protein.
Source sequence(s)	AC072018, AC112907, AK312868
Consensus CDS	CCDS3284.1
UniProtKB/TrEMBL	A8K660
UniProtKB/TrEMBL	B2R773

http://www.ncbi.nlm.nih.gov/nuccore/NM_001177800.1?&feature=CDS
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Nucleotide Search
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Send:

Homo sapiens adiponectin, C1Q and collagen domain containing (ADIPOQ), transcript variant 1, mRNA

NCBI Reference Sequence: NM_001177800.1

FASTA Graphics

Go to:

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  ORGANISM  Homo sapiens
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Articles about the ADIPOQ gene

Adiponectin and long-term mortality in cor [Arterioscler Thromb Vasc Biol. 2

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- Homo sapiens adiponectin, C1Q a collagen domain containing Nucleot
- Homo sapiens anti-Mullerian hormone (AMH), RefSeqGe Nucleot
- AMH anti-Mullerian hormone [Hom sapiens] Ge
- amh homo sapiens (15) Ge
- Homo sapiens adiponectin, C1Q a collagen domain containing Nucleot

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   1201 tcctgaggct gagagagtta agtgaatgtc taaggtcaca cagtattaag tgacagtgct
```

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Homo sapiens adiponecti... adiponectin precursor [Homo... Homo sapiens anti-Mullerian ...
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Homo sapiens adiponectin, C1Q and collagen domain containing (ADIPOQ), transcript variant 1, mRNA

NCBI Reference Sequence: NM_001177800.1

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```
LOCUS       NM_001177800            4629 bp    mRNA    linear   PRI 17-FEB-2013
DEFINITION  Homo sapiens adiponectin, C1Q and collagen domain containing
            (ADIPOQ), transcript variant 1, mRNA.
ACCESSION   NM_001177800
VERSION     NM_001177800.1  GI:295317371
KEYWORDS    .
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 4629)
```

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Articles about the ADIPOQ gene
Adiponectin and long-term mortality in cor [Arterioscler Thromb Vasc Biol. 2013]

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```
>gi|295317371|ref|NM_001177800.1| Homo sapiens adiponectin, C1Q and collagen domain
containing (ADIPOQ), transcript variant 1, mRNA
AGGCTGTTGAGGCTGGGCCATCTCCTCCTCACTTCCATTCTGACTGCAGTCTGTGGTTCTGATTCCATAC
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```

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Articles about the ADIPOQ gene

Adiponectin and long-term mortality in cor [Arterioscler Thromb Vasc Biol. 2013]

Genetic association of adiponectin with type 2 diabetes in Jordaniar [Gene. 2013]

Effects of omega-3 fatty acids supplementa [Asia Pac J Clin Nutr. 2012]

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Design at least one primer and/or *probe* which crosses one *exon junction*